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EFFECT OF SELECTED PLANT EXTRACTS ON POST-HARVEST FUNGAL ROT OF TOMATO (*Lycopersicum esculentum* Mill).

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ABSTRACT
Post harvest spoilage caused by fungal pathogens has been reported to reduce the quality of tomato fruits. Identifying these problems to prolong shelf life will make tomato available in Yola. Based on the foregoing, laboratory experiments were conducted in the Department of Plant Sciences of Modibbo Adama University of Technology, Yola. The experimental layout was a completely randomized design of two plant extracts. Each plant extract was at the same concentration. The result obtained showed that five species of fungi identified as *Rhizopus stolonifer*, *Fusarium oxysporum*, *Aspergillus niger*, *Fusarim solani* and *Geotrichum candidum* were associated with the spoilage of tomato fruits. All the fungi were found to be pathogenic on tomato in Yola. The efficacy of two plant materials namely *Moringa oleifera* and *Vernonia amygdalina* were investigated on the fungal isolates. Varying concentrations (40%, 60% and 80%) of ethanol leaf extracts were applied *in-vitro* on the fungal isolates and the mycelia growth inhibition was monitored. the highest inhibition caused by the ethanol extract of *M. oleifera* was on the growth of *F. oxysporum* (83.84%) and *V. amygdalina* was on the growth of *A. niger* (78.55%) followed by *G. candidum* (83.41%) and *F. oxysporum* (78.28%) respectively and the least mean percentage inhibition was recorded on the growth of *A. niger* (67.26%) and *R. stolinifer* (76.49%) respectively. The mean inhibition of *V. amygdalina* was higher (77.54) than that of *M. oleifera* (76.61). The findings from this study are significant as it contributes information on the post harvest pathology and management of tomato using plant extract.
Keywords: Tomato, post-harvest, fungal rot, plant extracts, ethanol extracts

INTRODUCTION

Tomato is perhaps the most important popular vegetable crop grown all over the country, both the wet and dry season cropping systems contribute immensely to the national requirement, but the bulk production is from the dry season cropping system grown yearly under irrigation in Nigeria (Ojo *et al*., 2009). Tomatoes are considered to be one of the most economically important crops of all those that exist in the world (Rahman *et al*., (2003). Tomatoes are also one of the main ingredients in hundreds of dishes and products that are sold in supermarkets throughout the developed world, this means that the demand of tomatoes from countries will be extremely high (Mourvaki *et al*., 2005).

Tomatoes are also a popular choice by people who wish to grow fruits and vegetables in their own gardens, not only can they be used raw in salads, but they are also an essential part of many recipes as well as many produced products such as tomato ketchup and chutney (Iheanacho, 2003). Tomato commercialization is limited by rotting caused by *Alternaria alternata* (Fr.:Fr.) Keissl. Or by *Botrytis cinerea* Pers.:Fr. (Jones et al., 1991). In developing countries, losses of fruits and vegetables during post- harvest fluctuate between 20 and 50% (Kader, 2002). It has been estimated that 20 - 50% of tomato fruits harvested for human consumption are lost through microbial spoilage. Other losses result from damage by dynamic stresses during transit, and through rough handling during loading and unloading (Aworth, 1985).

A number of economically important tomato diseases caused by fungi are transmitted by seeds or transplants (Ivanović and Mijatović, 2003). Tomatoes are parasitized by a number of pathogens, including *Fusarium oxysporum* Schlecht, the causal agent of fusarium wilt of tomato (Aleksić *et al*., 1990; Ivanović and Mijatović, 2003), which is one of the most

important species as tomato pathogen (Jones *et al*., 1982; Smith *et al*., 1988; Agrios, 2005). In an indoor environment due to high temperature and humidity, *F. oxysporum* can cause significant damage (Mijatović *et al*., 2007).

Fresh vegetable fruits can be contaminated with various fungi, including *Alternaria*, *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium* and *Trichoderma* species (Ivanović and Mijatović, 2003). These species can produce mycotoxins, contaminators of food, which is an important issue for human health (Ivanović and Mijatović, 2003). Post-harvest Fungal rot is one of the most common problems of fresh tomato fruits in storage and warehouses (Sharma, 1994).

Due to increased awareness about the risks involved in the use of synthetic pesticides, much attention is being focused on the alternative method of pathogen control, the spiraling up cost of chemical fungicides particularly in those countries where pesticides are important, pollution to soil, water, air by the accumulation of obnoxious chemicals residues due to continuous use of fungicides and development of resistance races to these chemicals are there, now forcing the scientists to look for methods which are ecologically friendly, safe and specific for pathogens.

The recurrent and indiscriminate use of fungicides have posed a serious threat to human health and to the existing human eco geographical conditions as some of those chemicals have already been proved to be either mutagenic, carcinogenic or teratogenic. Keeping in view the drawback of chemical management of plant, the use of plant extract in disease management is therefore gaining importance (Kiran *et al.* 2006; Okigbo, 2009). The objective of this study is therefore, to evaluate locally available plant extracts: *Vernonia amygdalina* and *Moringa oleifera* to control post-harvest tomato fruit rot pathogen.

MATERIALS AND METHODS

Source of Samples

Tomato fruits with symptoms of spoilage were randomly collected from the market stalls at Yola main market and Jimeta modern market on the 16 July, 2017. Fresh tomato fruits were also collected and packed into a sterilized polythene bag and taken to the laboratory for pathogenicity test on the 14 August, 2017.

Preparation of Potato Dextrose Agar (PDA)

Potato dextrose Agar (PDA) was used for the isolation of the pathogen of interest. The PDA was prepared according to the manufacturer's instruction; thereafter the media were autoclaved at 121°C for 15 minutes at 101Ibs pressure. After autoclaving three drops of streptomycin were incorporated into the sterilized media to prevent bacterial growth (Smith and Onion, 1983).

Isolation of the pathogens

Fruits exhibiting symptoms of spoilage were brought into the plant pathology laboratory for isolation of pathogens. Twenty (20) rotted fruits of tomato were washed with clean water and surface sterilized with 70% ethyl alcohol. A sterile scalpel was used to cut 3mm x 3mm sections of tissue from the tomato moving from the healthy portions to the diseased portion where the fungus is likely to be more active. Cut portions of tomato were aseptically placed on potato dextrose agar in Petri dishes and incubated for four (4) days at ambient temperature of 25°C ±3°C. The set up were observed for 7 days until the organisms became fully grown and covered the Petri dishes. Pure cultures of the individual isolates were made after series of inoculations into sterile Potato dextrose agar (Thomas, 1979).

Identification of isolated fungi

Microscopic examination was made after examining the colony characteristics on media. A sterile needle was used to take a little portion of the hyphae containing spores on to a glass slide which was stained with Lactophenol cotton blue and observed under the microscope for the structures of the fungi (Frazier, 1978). Morphological structures were observed under the microscope and compared with the structures in Alexopoulos *et al.* (2002) .

Pathogenicity Test

Fresh tomato fruits were washed with sterile distilled water, wiped dry using Whatman No.1 filter paper and surface sterilized with 0.1% mercury chloride solution to remove surface contaminants and rinsed in three changes of sterile distilled water. A sterile razor blade was

used to make a 2mm cut on the fruits and then culture of the isolates were introduced into the open cut surface and replaced with the core and sealed with Vaseline jelly. Fruits were inoculated in three replicates. The fruits were incubated for 5 days (Okigbo *et al.*, 2009). On establishment of disease symptoms, inocula from the infected fruits were taken and cultured until pure cultures were obtained.

Collection and Preparation of Leaf Extracts

Fresh leaves of *Vernonia amygdalina* and *Moringa oleifera* plants were collected from Sangere village, Girei local Government, Adamawa State on 21 August, 2017. These plants were taken to the Plant Science Department of Modibbo Adama University of Technology, Yola.

The collected plants were washed thoroughly under running tap water and were allowed to air dry for 7 days. These were ground separately. Thirty grams of each sample was added to 15ml of ethanol in separate conical flasks, this was vigorously shaken and left to stand for 24 hours. The samples were filtered with 3 layers cheese cloth and filtrate extract preparation of 80, 60 and 40% concentrations were used (Ijato *et al*., 2011).

Effect of Plant Extracts on Fungal Mycelia Growth

The effect of the extract on fungal growth was evaluated by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicates the centre of the plates. This was done before dispensing PDA into each of the plates (Ijato *et al*., 2011). The extracts were poured into the flask plugged with cotton wool and heated for about 10 minutes to avoid contamination (Madari and Singh, 2005). About 2 ml of the extract of *V. amygdalina* and *M. oleifera* were separately introduced into the Petri-dish containing the media and the pure isolate (poisoned food method), Control experiments were without addition of any plant extract but sterile distilled water. Fungi growth inhibition was determined in terms of percentage spore germination (Nene and Thalpiyal, 2000).

Inhibition Percentage (%) = DC- DT / DC X 100

Where: DC - Average Diameter of fungal spore germination in control

DT - Average diameter of fungal spore germination with treatment.

Data Analysis

The experimental layout was a completely randomized design of two plant extracts. Each plant extract was at the same concentration. The experiment was replicated three times. All the data were analyzed using analysis of variance (ANOVA) (Gomez and Gomez, 1984). Least Significant Different (LSD) was used to separate the means where there was significant difference (Scheff, 1953). The statistical package used to analyze the results was Statistics for Applied Sciences (SAS).

RESULTS

Isolation and Identification of Fungi

The fungal isolates from the tomatoes studied in this research which was found through pathogenicity test to be responsible for the post harvest rot of Tomato fruits in Yola were identified as *Rhizopus stolonifer, Fusarium oxysporum, Aspergillus niger, Fusarim solani* and *Geotrichum candidum.*

Pathogenicity Test

The result of the pathogenicity tests showed that all the isolated fungi were pathogenic on tomato fruits; from visual observation the symptoms were similar to those of the rotten tomato fruits from which the organisms were isolated. Under the microscope, the fungi re-isolated were the same as those used for the pathogenicity test. Also the wound created by the razor blade initiated spoilage as it formed opening for the easy penetration of the pathogens. *Rhizopus stolonifer* completely disintegrated the affected tissue (100% infection) with extensive *mycelia* growth covering the fruit and on PDA within 4-5 days. The rot induced by *Fusarium oxysporum and Fusarium solani* exhibited moderate to severe infection 4-7 days after inoculation. Water soaked lesions that spread rapidly covering the fruit was the usual pattern of pathogenicity. The fruit then became covered by white cottony mycelium topped by pinkish mass of fungal spore that completely disintegrated the tomato 7 days after inoculation. *Geotrichum candidum* infection became manifested 2 days

after inoculation and became severe 4-7 days later. This was characterized by whitish cheesy-like lesion on rotted fruits and on agar plate. Rot development on inoculated fruit was gradual. In all cases soft rot was induced that became watery accompanied by offensive odour.

Effect of ethanol plant extracts on isolated organisms

Analysis of Variance (ANOVA) on the effect of ethanol plant extracts on isolated organisms showed that all the means are highly significant ($P < 0.01$). Table 1 showed that the highest inhibition caused by the ethanol extract of *M. oleifera* was on the growth of *F. oxysporum* (83.84%) and *V. amygdalina* was on the growth of *A. niger* (78.55%) followed by *G. candidum* (83.41%) and *F. oxysporum* (78.28%) respectively and the least mean percentage inhibition was recorded on the growth of *A. niger* (67.26%) and *R. stolinifer* (76.49%) respectively. The mean inhibition of *V. amygdalina* was higher (77.54) than that of *M. oleifera* (76.61)

Table 1. Inhibition Effect of Ethanol Plant Extract on the Inhibition of Mycelial Growth of Fungi Pathogens (%)

Organisms	*Moringa oleifera*(%)	*Vernonia amygdalina*(%)
Geotrichum candidum	83.41[a]	76.60[c]
Aspergillus niger	67.26[c]	78.55[a]
Fusarium oxysporum	83.84[a]	78.20[a]
Fusarim solani	78.87[b]	77.89[b]
Rhizopus stolonifer	69.67[c]	76.49[c]
Mean	**76.61**	**77.54**
LSD	**2.23**	**2.47**
P- Value	**0.01**	**0.01**

Means followed by the same letter(s) are not significantly different at 1% significance level

Effect of ethanol plant extracts and concentration on isolated organisms

The interaction effect of ethanol plant extracts and concentration on isolated organisms showed that at 40% concentration both *Moringa oleifera* and *Vernonia amygdalina* had the lowest mean percentage inhibition of 68.03% and 68.59% and the highest mean percentage inhibition was recorded to be 76.61% and 77.54% at 80% concentration respectively, the mean inhibition of *V. amygdalina* was higher (77.54) than that of *M. oleifera* (76.61) at $P < 0.01$ all means are highly significant (Table 2).

Table 2. Effect of Ethanol Plant Extracts and Concentration on Isolated Organisms

Concentration (%)	*Moringa oleifera*	*Vernonia amygdalina*
40	68.03[c]	68.59c
60	75.84[b]	78.35[b]
80	85.96[a]	85.71[a]
Mean	**76.61**	**77.54**
LSD	**1.73**	**1.91**
P – Value	**0.01**	**0.01**

Means followed by the same letter(s) are not significantly different at 1% significance level

DISCUSSION

Five fungal species were found to be responsible for the post harvest rot of tomato fruits in Yola. These includes *Rhizopus stolonifer, Fusarium oxysporum, Aspergillus niger, Fusarim solani* and *Geotrichum candidum.* This is in agreement with the report that species of *Penicillium, Aspergillus* and *Mucor* are common post-harvest fungal pathogens of tomatoes (Liu and Ma, 1983), it is also in agreement with the report that *Aspergillus niger*, *Mucor,* and *P. digitatum* were found to cause rot of cassava (Ijato *et al*., 2011). It also agrees with the report that *Alternia* and *Rhizopus* were the major causes of post-harvest losses of tomato and grey mould (Uke *et al*., (2012) and also *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, G*eotrichum candidium* were isolated from infected tomato fruit parts in Ado-Ekiti (Ijato *et al*., 2011). *Helminthosporium solani,*

Aspergillus niger, Penicillium digitatum and Mucor piriformis were reported to be responsible for post harvest rot of tomatoes in Nsuka, *Fusarium moniliforme, Rhizopus stolonifer and Geotrichum candidum* were also reported to be associated with the spoilage of tomato fruits (Chuku *et al*., 2010).

Ethanol leaf extracts of *M. oleifera* and *V. amygdalina* inhibited the growth of the pathogens. Higher concentrations exhibited more inhibition than lower concentrations. This result is in line with the report that aqueous extract of *V. amygdalina* (80%) had higher inhibitory effect against *Geotrichum candidium* and *Aspergillus niger* than 60% aqueous concentration, similarly, 30% extract of *V. amygdalina* inhibited *Fusarium oxysporum* more than 20% (Ijato *et al*., 2011). It is also in agreement with the report that at higher quantity (3g), Ginger and Garlic had higher inhibition percentage of fungal mycelial growth than at lower quantity of 1gram (Chuku *et al*., 2010).

Ijato *et al.* (2011) reported that the inhibitory effect of the plant extracts could be alluded to the presence of antimycelia substances. Greater inhibition of fungal growth was observed at higher concentrations of the aqueous and extracts. *R. stolonifer, F. oxysporum*, F. solani, *G. candidium* and *A. niger* are common pathogenic fungi which cause tuber rot, fruit and vegetable rot. The results of the present investigation are vivid indications for the potential of plant extracts to control fungal pathogens.

It is also clear from the result that both the test plant extracts significantly reduce the radial growth of isolated fungi. It seems that the antifungal and the antimicrobial effects are the results of many compounds acting synergistically (Bangamboula *et al.* 2004).

CONCLUSION

This work has shown that the pathogens responsible for the post harvest rot of tomato fruits in Yola were *Rhizopus stolonifer, Fusarium oxysporum, Aspergillus niger, Fusarim solani* and *Geotrichum candidum. Rhizopus stolonifer* was found to be the most virulent pathogen and the least was *Geotrichum candidum*. Ethanol leaf extracts of *Moringa oleifera* and *Vernonia amygdalina* were found to be effective in inhibiting the mycelial growth of the pathogens and higher concentrations (80%) of the plant extracts exhibited more inhibition 85.96% and 85.71% respectively than lower concentrations (40%) 68.03% and 68.59% and is therefore recommended for fungal control in tomato since it is readily available, cheap and environmentally friendly.

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